# UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 09/918,242 | 07/30/2001 | Stephen C. Ekker | 09531-033001 | 2274 |

26191 7590 05/19/2004

FISH & RICHARDSON P.C.
3300 DAIN RAUSCHER PLAZA
60 SOUTH SIXTH STREET
MINNEAPOLIS, MN 55402

| EXAMINER | |
|---|---|
| ANGELL, JON E | |
| **ART UNIT** | **PAPER NUMBER** |
| 1635 | |

DATE MAILED: 05/19/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/918,242 | EKKER ET AL. | |
| | Examiner | Art Unit | |
| | J. Eric Angell | 1635 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *15 March 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *98-105* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *98-105* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *30 July 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *attached*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

### *Continued Examination Under 37 CFR 1.114*

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 3/15/04 has been entered.

2. This Action is in response to the communication filed on 3/15/04. The amendment has been entered. Claims 1-97 are now all cancelled. New claims 98-105 have been added. Claims 98-105 are currently pending in the application and are examined herein.

3. Applicant's arguments are addressed on a per section basis. The text of those sections of Title 35, U.S. Code not included in this Action can be found in a prior Office Action. Any rejections not reiterated in this action have been withdrawn as being obviated by the amendment of the claims and/or applicant's arguments.

### *Claim Rejections - 35 USC § 112, second paragraph*

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 98-105 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

6. Claim 98 recites the limitation "said selected nucleic acid" in lines 5-6. There is insufficient antecedent basis for this limitation in the claim, thus making the claim indefinite. Furthermore, claims 99-105 all depend on claim 98, and must encompass claim 98 in its entirety. As such claim 99-105 are also rejected for the insufficient antecedent basis for the limitation "said selected nucleic acid". It is noted that amending the claim to recite "a selected nucleic acid that is expressed during zebrafish embryonic development" would obviate this rejection (see scope of enablement rejection below).

***Claim Rejections - 35 USC § 112, first paragraph***

7. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. Claims 98-105 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The instant claims are drawn to methods of producing a zebrafish embryo comprising a polynucleotide analogue (wherein the polynucleotide analogue can be a morpholino, aminoamidate, etc, see claim 1), wherein the polynucleotide is specific for a selected gene of interest, wherein the method comprises administering the polynucleotide analogue to a zebrafish embryo such that it inhibits the expression of the selected gene of interest in larval and post-

hatching stages in development. It is respectfully pointed out that the claims encompass inhibiting the expression of a very large number of selected genes of interest, considering that the selected gene of interest can be any gene expressed during embryonic development, or a gene that is not expressed until post-embryonic development, including genes that are only expressed during larval and post-hatching stages of development.

The Written Description Guidelines for examination of patent applications indicates, "the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, or by disclosure of relevant, identifying characteristics, i.e. structure or other physical and/or other chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show applicant was in possession of the claimed genus." (See MPEP 2100-164)

In the instant case, the claims encompass a very large (possibly millions) of selected genes of interest, considering every zebrafish gene encompassed by the broad claims. The specification has only identified specific genes which are expressed at some point during zebrafish embryonic development. The specification has not identified any selected genes that are not expressed during zebrafish embryonic development that can be effectively targeted using the claimed method. The specification has only described a representative number of zebrafish genes that are expressed during embryonic development that are effective targets. Thus, limiting the claims to target genes that are expressed during zebrafish embryonic development would obviate this rejection. However, since the claims encompass genes which are not adequately

described by the specification (including genes that are not expressed during zebrafish embryonic development) the instant rejection is appropriate.

9. Claims 98-105 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

> "A method for producing a zebrafish embryo comprising a polynucleotide analogue wherein said polynucleotide analogue is selected from the group consisting of: a morpholino-modified polynucleotide, a 3'-5' phosphoroamidate, a peptide nucleic acid, and a polynucleotide comprising a ribose moiety having a 2' O-methyl group; wherein said method comprises contacting said zebrafish embryo, or an egg giving rise to said zebrafish embryo, with said polynucleotide analogue in an amount effective to reduce the expression of a selected nucleic acid that is expressed during embryonic development of said zebrafish embryo."

does not reasonably provide enablement for the full scope encompassed by the claims. For instance, the claims are not enabled for producing a zebrafish embryo comprising a polynucleotide analogue wherein the polynucleotide analogue reduces expression of any gene other than one that is expressed during embryonic development, or wherein the reduction in expression of the selected nucleic acid persists to larval or post-hatching stages. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make/use the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988).

*Wands* states on page 1404,
"Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention

The instant claims are drawn to a method of making a zebrafish embryo comprising a polynucleotide analogue by administering the polynucleotide analogue to a zebrafish embryo wherein the polynucleotide analogue is present in an amount effective for reducing the expression of a selected nucleic acid in said embryo and wherein the reduction in said expression of said nucleic acid persists to larval or post-hatching stages of development. As such, the claims encompass reducing the expression of any target gene in a zebrafish embryo such that the reduction in gene expression persists until after the zebrafish embryo hatches.

The breadth of the claims

The instant claims are very broad with respect to the selected nucleic acid (i.e, the nucleic acid that is the target for reduced expression). It is noted that the instant claim, as currently written, encompasses administering a polynucleotide analogue to an embryo wherein the polynucleotide analogue reduces expression of a gene that is not expressed during the embryonic stages of development. For instance, the claims encompass administering a polynucleotide analogue to a zebrafish embryo in an amount effective to reduce expression of gene that is expressed after the embryonic stage of the zebrafish (such as a zebrafish gene that is only expressed in adult zebrafish). Furthermore, the instant claims are very broad with respect to the

length of time for which the duration of reduced expression is desired. For instance, the instant claims encompass reducing the expression of the selected nucleic acid such that the reduction in expression persists to the larval or "post hatching stages of development". As written the claims encompass reducing expression until a stage that is long after the post hatching stage, such as the juvenile to adult development stage.

The unpredictability of the art and the state of the prior art

As mentioned above, the claims encompass a method for reducing the expression of a target gene in a zebrafish embryo. Methods of inhibiting the expression of a target gene, in general, where known in the art. For instance, methods of inhibiting target gene expression using antisense oligonucleotides that were complementary to the target gene mRNA were well known in the art. The vast majority of antisense methods were used as therapeutic methods to reduce the expression of the target gene for therapeutic effect, such as reducing the expression of a gene that was overexpressed in diseased cells. With respect to reducing gene expression during embryonic development, the prior art indicates that antisense technology was used to inhibit target gene expression in frogs as well as at least two specific example of inhibiting target gene expression in zebrafish (see Barabino, 1997, previously cited; and Roth, Development, 1999). However, with respect to the inhibition of zebrafish embryonic gene expression, the prior art does not indicate that the antisense molecules incorporated any chemical or structural modifications (such as morpholino, aminoamidate, 2' O-Me, etc.). Barabino teaches that an unmodified antisense RNA oligonucleotide which specifically hybridizes to the zebrafish Chx10 gene and can be used to reduce Chx10 gene expression in zebrafish embryos by processing the embryos and adding the antisense oligonucleotides to the embryo culture media. Barabino

teaches that the antisense method most likely only affects the early function of the genes because once the oligonucleotide is inside the cell it has only a limited stability and is eventually degraded (e.g., see p. 141).

With respect to the antisense polynucleotide analogues encompassed by the claims, the relevant art indicates that there are a number of characteristics relevant to the instant rejection. For instance, Heasman (Developmental Biology, 2002) teaches, with respect to morpholino modified antisense molecules,

> "Since morpholino oligos are DNA analogs, they are not susceptible to enzymatic degradation, unlike DNA oligos, and thus have much higher biological stability... Presumably, oligos eventually lose effectiveness by dilution. Clearly, the length of the loss-of-function effect of an injected oligo depends on the transcription and translation characteristics of the targeted mRNA, as well as the dose of the oligo, and will need to be determined for each gene of interest." (See p. 211, second column).
>
> "[M]orpholino oligos cannot simply be assumed to be consistently effective. In those cases where more than one oligo has been designed to be complementary to different parts of the 5' UTR sequence of a target mRNA, the oligos have had different degrees of effectiveness in blocking translation. This suggests that, as for DNA oligos, we are ignorant of many of the variables underlying morpholino activity." (See page 212, first column).
>
> "Several authors report that, at the high end of [the morpholino concentration] range, non-specific effects occur. These include widespread cell death... defects in epiboly... and neural degeneration..., effects that would not be expected from genetic mutants in which the genes are completely inactivated... Why morpholinos cause side effects is a major question that remains to be resolved. Clearly, the likely possibilities are either there are non-specific effects of morpholino oligos or contaminants, or that there are effects due to unexpected complementarity of the oligo to other unknown genes. The appearance of these effects is highly oligo dependent." (See page 211, first and second columns).

Thus, it is apparent that a modified antisense oligonucleotide, although it may be resistant to degradation, is still subject to decreased effectiveness due to increased dilution of the oligo over time. That is, as the organism grows, the oligo will become more dilute and become less

effective. The above citation also indicates that increasing the concentration of the modified oligonucleotide can result in non-specific effects. Thus, one of skill in the art would recognize that merely increasing the concentration of the oligonucleotide administered to the embryo would not necessarily overcome the loss of effectiveness associated with the dilution effect, and may, in fact, result in undesired non-specific effects.

It is noted that the prior art indicates that antisense oligos are subject to degradation and modified oligos are subject to the dilution effect—that is, as the embryo grows, the oligos become more diluted and less effective. There is no indication in the prior art that an antisense oligonucleotides (including polynucleotide analogues such as morpholinos) can be administered to a developing embryo (such as a zebrafish embryo) and be effective in reducing the expression of a gene that is expressed after embryonic stages of development. For instance, there is no indication that an antisense oligonucleotide can be administered to a zebrafish embryo and inhibit the expression of a gene that is expressed in larval or post-hatching stages. Considering the teaching in the art that the oligos are subject to the dilution effect, it is considered unpredictable that the claimed oligos could inhibit the expression of such genes.

Working Examples and Guidance in the Specification

It is acknowledged that the specification has working examples where morpholino-modified antisense oligos and peptide nucleic acid (PNA) modified antisense molecules specific for certain genes expressed during embryonic development (specifically: oep, chordin, ntl, twhh, shh, vegf, zfz2, chd, and tsg—see working examples and figures) were effective for reducing the expression of the target genes for 48 hours, when the oligos were administered to a zebrafish embryo. However, the duration of reduced expression does not appear to be assayed beyond 48

hours of development. Considering the teaching in the prior art that the oligos are subject to the dilution effect, it is unpredictable that the oligos administered to the zebrafish embryo would be able to inhibit expression of a gene that expressed after hatching (or in the larval stages of development).

Quantity of Experimentation

As indicated above, the claims encompass administering a polynucleotide analogue to a zebrafish embryo and inhibiting the expression of the target gene throughout and past embryonic development, including into the larval and post-hatching stages. Considering the unpredictable nature of the broadly claimed invention, additional experimentation would be needed in order to be able to practice the invention to the full scope encompassed by the claims. For instance, additional experimentation would be required to show that the polynucleotide analogues could be administered to a zebrafish embryo and reduce the expression of a post-embryonic gene. Furthermore, additional experimentation would be required to show that administration of the polynucleotide analogue to a zebrafish embryo would inhibit the expression of the target gene throughout embryonic development and even past embryonic development into the larval and post-hatching stages of development.

Level of the skill in the art

The level of the skill in the art is deemed to be high.

Conclusion

Considering the high degree of unpredictability recognized in the relevant art, the breadth of the claims, the limited of working examples and guidance in the specification, and the high

degree of skill required, it is concluded that the amount of experimentation required to perform the broadly claimed invention is undue.

***Response to Arguments***

The rejection of claims under 35 USC 102 are now moot as the instant claims are drawn to a method of making a zebrafish embryo comprising a polynucleotide analogue that is any one of: a morpholino-modified polynucleotide, a 3'-5'phosphoroamidate, a PNA, and a polynucleotide comprising a ribose moiety having a 2'-O-Me group. As the prior art does not teach a zebrafish embryo comprising any of these polynucleotide analogues, the rejection is withdrawn. Furthermore, the closest prior art (Barabino, previously cited) teaches a zebrafish embryo that comprises an antisense polynucleotide, but not a polynucleotide analogue. Furthermore, Barabino teaches that the antisense polynucleotide successfully inhibited the expression or the target gene, and also indicated that their method of administering the antisense oligos to the zebrafish embryo was a better method than directly injecting the embryo with the antisense oligonucleotide (e.g., see p. 141, second column). Therefore, one of ordinary skill in the art would not have been motivated to modify the antisense of Barabino such that it comprised any of the polynucleotide analogue modifications encompassed by the claims.

***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to J. Eric Angell whose telephone number is (571) 272-0756. The examiner can normally be reached on M-F (8:00-5:30) with every other Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John L. LeGuyader can be reached on (571) 272-0760. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Jon Eric Angell, Ph.D.
Art Unit 1635

DAVE T. NGUYEN
PRIMARY EXAMINER